

REMARKS

Claim 26 has been canceled. Claim 27 has been rewritten as an independent claim. Claims 28, 30 and 31 have been amended for clarity or to split claims reciting Markush groups into separate claims. New claims 60-85 have been added. The amendments and new claims are supported throughout the application, for example, at page 2, line 16, to page 6, line 30. No new matter has been added.

Upon entry of this amendment, claims 27-38 and 60-85 will be pending. Claims 27-31 and 60-85 are under examination.

The present claims relate to a method of producing a protein in a subject. The method includes introducing into the subject an immunologically privileged intermediate lobe (IL) pituitary cell that has been genetically engineered to express a protein. Applicants have found that IL cells are resistant to immune rejection when implanted in a host subject.

Rejections Under 35 U.S.C. §112, Para. 1

Claims 26-31 are rejected as "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." In particular, the Examiner provides the following grounds for the rejection.

The specification must be enabling for therapeutic transplantation, as this is the sole asserted utility for the claimed method. The specification does not offer specific guidance as to how the claimed method could be used therapeutically for any disorder. No working examples demonstrate a therapeutic effect upon transplantation of the cells recited in the claims. The specification fails to provide specific guidance relating to the amount of cells to inject, the site of injection, and extent of cellular persistence, required to provide any therapeutic benefit for any disorder.

The claimed methods encompass *ex vivo* gene therapy. However, gene therapy is not routinely successful. (...) The specification lacks any working examples showing that the claimed cells, once delivered to an appropriate site, would express the exogenous gene at a level sufficient to provide adequate product to

effect the desired therapy in an immunocompetent animal. At the time the application was filed, the art of administering any type of genetic expression vector to an individual so as to provide a tangible therapeutic benefit was poorly developed and unpredictable.

This rejection has been met by narrowing the scope of the cell recited in the broadest claim to an intermediate lobe (IL) pituitary cell. Accordingly, claim 26 has been canceled and claim 27 has been rewritten as an independent claim. Applicants submit that the present claims fully satisfy the enablement requirements of 35 U.S.C. §112. First, Applicants provide ample teachings, including an *in vivo* working example, to show that the claimed methods work exactly as claimed. Second, the Examiner's concerns regarding the state of gene therapy in the prior art are not applicable to the presently claimed methods.

I. Applicants' disclosure shows that the claimed methods work exactly as claimed

Contrary to the Examiner's statements, Applicants do provide working examples of the claimed methods. In particular, the Examiner is directed to Example 6 of Applicants' disclosure. As shown by the passage below, Example 6 describes the transplantation of cells genetically engineered to express insulin into diabetic nongobese diabetic (NOD) mice, a well known and widely accepted, established animal model system for diabetes. NOD mice are immunocompetent. Thus, the example (and other sections of the specification discussed below) provides a working example in which genetically engineered IL pituitary cells from one animal were transplanted into an immunocompetent donor. Sufficient insulin was produced and released such that the donor showed, in the words of the specification, "complete remission," "return to near-normoglycemia" and insulin levels "in a similar range to random insulin levels of non-diabetic control mice." To wit:

[T]ransplantation of 4 intermediate lobe pituitaries under the kidney capsule of spontaneously diabetic NOD mice resulted in a significant gain in body weight (Fig 3A) and in the complete remission from diabetic symptoms. This was associated with the progressive return to near-normoglycemia (Fig 3B), with mean BG levels decreasing from 484 ± 21 mg/dl pre-transplantation to 150 ± 43 mg/dl after transplantation (n=6). In parallel with this drop in BG, random insulin levels increased from 4 ± 0.2 μ U/ml pre-transplantation to 42 ± 9 μ U/ml post-transplantation, in a similar range to random insulin levels of nondiabetic control

mice [$39 \pm 9 \mu\text{U}/\text{ml}$ ($n=6$)]. At the end of the transplantation period, immunohistochemistry of the grafts of the recipients showed abundant insulin staining with no evidence of lymphocytic infiltration. Similar analysis of pancreatic sections from recipients did not reveal any significant insulin-positive cells, confirming that the enhanced insulin levels post-transplantation were due to the transgenic tissue implants. These results demonstrate that the intermediate pituitary-derived insulin is biologically active and are consistent with the biochemical studies which showed that the great majority of insulin secreted by the transgenic pituitaries is fully processed, mature insulin. Diabetic NOD mice receiving nontransgenic (control) intermediate lobe pituitaries had no reduction in serum BG levels, and had increasingly severe diabetic symptoms which resulted in their demise within 3 weeks after transplantation. (Paragraph bridging pages 25-26 of specification, emphasis added)

In view of this example alone, the Examiner's statement that "the specification lacks any working examples showing that the claimed cells, once delivered to an appropriate site, would express the exogenous gene at a level sufficient to provide adequate product to effect the desired therapy in an immunocompetent animal" is clearly incorrect, since, as is known in the art, NOD mice are immunocompetent. Indeed, the use of NOD mice is a particularly stringent test for tolerance of the IL cells because NOD mice have an immune system that is hyperresponsive to attacking self as well as foreign tissues. Moreover, the inventors have shown that IL cells from a CH3 mouse strain are not rejected by allogeneic BALB/c mice (a different strain of mouse). See, e.g., Example 25 at page 43 of the specification. These results clearly show that the presently claimed methods work as claimed.

Further, additional guidance on making and evaluating expression of genetically engineered IL pituitary cells is provided, e.g., at page 2, line 28 to page 3, line 13; page 13, line 29 to page 14, line 12; additional procedures for harvesting and transfecting IL cells are described, e.g., at page 3, lines 19-22 and Examples 10 and 11 (pages 30-31). With regard to the Examiner's concern about specific guidance relating to "the site of injection, and extent of cellular persistence" Applicants note that special sites of injection and methods of coating or otherwise protecting cells to increase persistence in the host are generally employed in cell therapy because of problems with adverse host immune responses to the implanted cells. Importantly (and as discussed in more detail below), Applicants' invention directly addresses this issue by the use of immunologically privileged cells (IL cells) as a vehicle for protein

delivery. I.e., Applicants have shown precisely that IL pituitary cells can evade the host immune system. Thus, the parameters of the site of injection, and extent of cellular persistence discussed by the Examiner are less critical in the claimed methods than in other cell therapy methods. Applicants have shown that autologous and allogeneic IL cells evade immune attack by the host subject. Nonetheless, guidance regarding methods of implanting cells, the site of injection, and extent of cellular persistence is provided at, e.g., page 7, lines 19-20; page 8, lines 24-30, of the specification.

Although insulin is provided as an exemplary protein that can be produced using the claimed methods, the methods are intended generally as a means for delivering a protein to a subject. The prior art contains numerous examples of proteins that can usefully be delivered. At least the following were well documented in the prior art at the date of priority: the use of glucocerebrosidase for treatment of Gaucher's disease (Harrison's Principles of Internal Medicine, 12th ed., Eds. J.D. Wilson et al., McGraw-Hill, Inc., NY, 1991, p. 43); the use of growth hormone to treat growth hormone deficiency (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, p. 1342); interferon therapy (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, pp. 1190-1191), tissue plasminogen activator therapy (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, pp. 1323-1324), calcitonin therapy (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, p. 1509), colony stimulating factor therapy (Goodman and Gilman, The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press, 1990, pp. 1281-1282)¹. The known methods for administering these proteins include systemic and local administration, either of which is possible using the claimed methods. Given the knowledge and the tools (e.g., cloned genes) in the prior art relating to these and other proteins that can be administered using the claimed methods, any experimentation required is merely routine.

In sum, the inventors have introduced into a subject an immunologically privileged intermediate lobe pituitary cell that has been genetically engineered to express an exemplary protein (insulin) and the protein was provided to the host subject *in vivo*. Given the guidance in

¹ These references are provided in a supplemental information disclosure statement filed under separate cover.

the specification and the knowledge and skill in the art, there is no reason whatsoever to expect that the presently claimed methods would not work for other proteins exactly as described.

II. The problems of gene therapy argued by the Examiner do not apply to the present claims

The rejection for lack of enablement is based in large part on certain publications which, according to the Examiner, express the opinion that the field of gene therapy in general is beset by problems and has not demonstrated clinically effective results. In support of the rejection, the Examiner cites the NIH ad hoc panel report on gene therapy (Orkin et al.); a review of gene therapy largely focusing on gene therapy vectors (Friedmann); and a Nature News and Views Feature (Verma et al.). Verma et al., similarly to the other two references, summarizes the most "formidable challenges" of gene therapy as "lack of efficient delivery systems, lack of sustained expression, and host immune reactions." (Verma et al., page 329, first paragraph). However, for at least the following reasons, these concerns do not apply to the pending claims and not render the claimed methods unpatentable.

A. The claimed methods do not involve the direct administration of a gene or vector

The problems with gene therapy discussed in the cited references relate in large part to problems with directly delivering a gene (DNA) or a gene bearing vector to an individual. *In vivo* gene therapy generally requires that DNA be administered directly to the patient. The DNA must then reach the target cells within the patient, and be incorporated into an adequate number of target cells. The Examiner asserts that "administering any type of genetic expression vector to an individual so as to provide a tangible therapeutic benefit was poorly developed and unpredictable." Verma et al. state, "The Achilles heel of gene therapy is gene delivery." However, the present method of cell therapy does not involve the direct administration of a gene or vector (DNA) to an individual. In contrast, the present method permits manipulation of a specific type of cell (*IL* cells) *in vitro* to produce a final population of cells selected to ensure not only incorporation of the DNA, but also expression of the protein at a desired level. The rate of protein production by the cells can be ascertained *in vitro*, so that the appropriate number of cells producing the desired amount of the protein can be determined prior to implantation in the

patient. Thus, low efficiency can be overcome with *in vitro* transfection and selection techniques. Guidance in this regard is provided, e.g., at page 2, line 28 to page 3, line 13, and page 13, line 29 to page 14, line 12. Indeed, Example 6 of Applicants' disclosure shows that genetically engineered cells obtained from transgenic animals do stably express the desired protein when implanted *in vivo*. Examples 7 and 11 of Applicants' disclosure show that cultured IL cells are efficiently transduced by viral vectors. Moreover, methods of genetically engineering cells using non-viral methods for stable expression for *ex vivo* therapy were known at the date of priority (see, e.g., U.S. Patent 5,641,670). Thus, the vagaries of direct gene delivery and stable expression associated with *in vivo* gene therapy that may render it difficult and unpredictable do not apply in the present case.

B. The claimed methods avoid problems with immune rejection

The cited references emphasize that one of the most problematic aspects of gene therapy is the occurrence of adverse host immune reactions. Orkin et al states that gene therapy "should be accomplished without adverse reactions from the recipient" (page 7). Friedmann remarks that even when cells are effectively transfected *in vivo*, "soon host defenses come into play, killing the altered cells and inactivating their new genes." Verma et al. similarly are concerned that "host immune reactions--remain formidable challenges." In stark contrast, Applicants' methods employ IL pituitary cells which are immunologically privileged. Applicants have shown that these cells do not trigger the normal immune response when introduced into a subject even if the cell is from a genetically different donor (see Example 25). Therefore, this particular problem of gene therapy in the prior art is precisely one that Applicants' invention can directly address. This is clearly emphasized in the summary of the results provided by Applicants' disclosure at page 9, line 15 to page 10, line 18, as follows:

1. When implanted under the kidney capsule of spontaneously diabetic NOD mice, the insulin-producing IL pituitary cells did not become infiltrated by the cells of the immune system while islets implanted under the opposite kidney capsule were rapidly targeted and destroyed;

2. Insulin-producing IL tissues cured diabetes when transplanted into spontaneously diabetic NOD mice. Examination of the IL grafts at the end of transplantation period showed healthy grafts that were devoid of evidence of

immune system attack and that contained abundant amounts of insulin. In contrast, diabetic mice similarly transplanted with insulin-producing islet grafts developed recurrence of their diabetic symptoms with the islet grafts becoming severely infiltrated by cells of the immune with complete destruction of the insulin-producing β cells;

3. When islet and insulin-producing IL cells were mixed together and co-transplanted into diabetic NOD mice, severe lymphocytic infiltration developed over the entire graft, including areas containing IL cells. Although the insulin-producing islet β -cells were completely destroyed, the insulin-positive IL cells remained intact. Indeed, all of the insulin-positive cells that remained in the grafts at the end of the transplantation period colocalized with POMC-peptides and were thus pituitary-derived. Thus, even when placed in direct contact with diabetogenic infiltrates, the ILins cells were resistant to autoimmune-mediated cell destruction;

4. In other experiments, streptozotocin-induced diabetic *scid* (severe combined immuno-deficient) NOD mice were transplanted with similar amounts of insulin-producing tissues (110 islets and 2 ILins pituitaries) under the opposite kidney capsules. When their immune system was reconstituted with 20×10^6 cells of a highly pathogenic insulin-specific T cell clone (PD-12-4.4), they developed recurrence of their diabetic symptoms. As expected, their islet grafts became severely infiltrated with loss of the insulin-producing β cells. In contrast, examination of the insulin-producing IL grafts transplanted under the contralateral kidney capsule of these mice showed no evidence of infiltration by these clones, even though they are specifically reactive to insulin. Indeed, the ILins grafts in *scid*/NOD mice that received the insulin-specific "killer" T cells contained abundant insulin-staining cells, similar in appearance to ILins grafts of control *scid*/NOD mice that only received saline.

These results demonstrate that the IL pituitary cells can evade recognition and destruction by autoaggressive cells of the immune system in IDDM. The protection of IL from autoimmune recognition is a unique feature that is clearly advantageous, especially for transplantation purposes.

As can be seen from the results summarized above, the claimed methods avoid problems with immune rejection that can be seen in other cell transplantation methods. A particularly rigorous test of immune tolerance to the IL cells is presented in item 4 as summarized above. In this experiment, the immune system of immunodeficient animals (*scid*-NOD mice as opposed to regular, immunocompetent NOD mice used in other experiments) was reconstituted with 20

million insulin-specific cytotoxic T cells (creating a mouse in which the T cells consisted entirely of pathogenic T cells). As reported by Applicants, when the mice were rendered diabetic and transplanted with insulin-producing cells, the islet grafts were destroyed. However, the insulin producing IL grafts under the opposite kidney capsule were intact and healthy and continued to produce insulin. Clearly, this is a very rigorous test of immune tolerance to the genetically engineered IL cells.

In view of the above, the teachings about unpredictability or poor results one may or may not be able to derive from the cited publications apply solely to *in-vivo* gene therapy (direct administration of DNA or viral vectors), or to *ex-vivo* gene therapy/cell therapy with cells that induce a host immune response. The teachings of the cited references do not apply to cell therapy methods as presently claimed.

Thus, as discussed above, by omitting both sources of problems (the direct administration of DNA and the adverse host immune response), Applicants avoid the pitfalls attributed to genetic therapy by the cited references. These pitfalls do not apply to the present invention, which is limited to cell therapy using immunologically privileged cells. Thus, there is no reason whatsoever to expect that the presently claimed methods would not work exactly as described.

Finally, Applicants note that the statements from the cited references quoted in the office action all are directed to the lack of positive endpoints in clinical efficacy in gene therapy trials. As the Examiner knows, Phase I stage clinical trials (which defined the vast majority of gene therapy trials at the time of filing) are done on the sickest patients, and are designed to evaluate tolerance and safety, not clinical efficacy. Even Orkin acknowledges that “because these studies have not been designed to measure efficacy, they do not include sufficient controls to evaluate the true merits of gene therapy or compare this approach with conventional approaches” (Orkin, page 13, emphasis added). Thus, the fact that “clinical efficacy” or “improved health” had not been proven by gene therapy trials at the time of filing is not dispositive of the feasibility of gene therapy in general, much less of the feasibility of Applicants’ method. Moreover, Applicants note that while therapeutic efficacy in clinical trials may be a requirement for obtaining regulatory approval to practice a therapeutic method, it is certainly not a requirement for patentability. Applicants have shown that the claimed methods work in whole animals to

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provide a protein *in vivo* and produce a therapeutic effect. Nothing more is required under U.S. law.

Accordingly, Applicants respectfully request that the rejection be withdrawn.
